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The effect of the apolipoprotein E phenotype on plasma lipids is not influenced by environmental variability: results of a Dutch twin study

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Abstract. We tested the influence of the apolipoprotein E (apoE) polymorphism on the intrapair differences in the levels of plasma cholesterol, plasma triglycerides, low density lipoprotein-cholesterol, apoB and apoE in monozygotic (MZ) twins, and estimated whether or not there was a interaction between the apoE polymorphism and environmental factors. In 65 MZ twin pairs, the intrapair differences in the measured lipoprotein parameters were similar in the different apoE phenotype classes. This indicates that the effect of the apoE polymorphism is not influenced by environmental variability between the MZ pair members and accordingly identifies the APOE gene as a "level" gene.

Introduction

Apolipoprotein E (apoE) is one of the major protein constituents of chylomicron and very low density lipoprotein (VLDL) remnants. It plays a central role in the receptor-mediated uptake of these particles by acting as a high affinity ligand for hepatic lipoprotein receptors (Sherril et al. 1980; Weisgraber et al. 1982). ApoE also plays a key role in the conversion of VLDL via intermediate density lipoprotein (IDL) into low density lipoproteins (LDL) (Demant et al. 1991). Human apoE can be separated by isoelectric focusing into three major isoforms, E2, E3 and E4, which each differ in their isoelectric point by a single charge unit, apoE4 being the most basic and E2 the most acidic form. These isoforms are encoded by three codominant alleles. E*2, E*3 and E*4, at a single APOE gene locus on chromosome 19 (Zannis and Breslow 1981; Scott et al. 1985). ApoE3 is the most common isoform. ApoE4 differs from apoE3 by an arginine for cysteine substitution at amino acid residue 112 [designated as apoE4 (Cys112→Arg)], whereas apoE2 differs from apoE3 by a cysteine for arginine substitution at residue 158 [apo E2(Arg158→Cys)]. Various population studies have demonstrated an influence of the apoE polymorphism on plasma lipid and (apo)lipoprotein levels (reviewed by Davignon et al. 1988). In these studies, the APOE*2 allele is associated with lower levels of total plasma cholesterol, LDL cholesterol and apoB, whereas for the APOE*4 allele, the opposite holds true. The apoE polymorphism was found to explain between 1.4% and 8.7% of the interindividual variability of plasma cholesterol (Smit et al. 1988; Boerwinkle and Utermann 1988).

There are a number of reports showing an interaction between the apoE polymorphism and environmental factors (e.g. diet) in their effect on lipids and (apo)lipoproteins (for recent reviews on this, see Abbey et al. 1991; Ferrel 1992). An approach that can be used to study this interaction is that based on studying monozygotic (MZ) twin pairs, as outlined by Magnus et al. (1981) and Berg (1984, 1987, 1990). MZ twins share all nuclear genes; thus, the difference in a quantitative trait, e.g. plasma cholesterol, observed between the two members of an MZ twin pair must reflect variation caused by the environment, such as diet. In order to study the effect of the apoE polymorphism, we have grouped MZ twin pairs according to apoE phenotypes. This makes it possible to determine whether a group of MZ twins with a specific apoE phenotype has a significantly larger or smaller mean [absolute] intrapair difference in plasma cholesterol than other groups of MZ pairs with other apoE phenotypes. The finding of a significant and consistent effect would imply the existence of an interaction between the APOE gene and environmental factors. Accordingly, in the present study, we estimated the influence of the apoE polymorphism on the intrapair differences of plasma lipids in 65 MZ twin pairs, as part of a study of 160 Dutch twin families.

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Materials and methods

Population description and sample collection

The individuals described in this article were apparently healthy, and belonged to a cohort of 160 twin families (158 fathers, mean age 48.1 years, SD = 6.7; 160 mothers, mean age 45.6 years, SD = 5.9; 161 boys, mean age 16.7 years, SD = 1.8; 159 girls, mean age 16.7 years, SD = 2.2). All twins were still living with their parents. The addresses of the families were obtained from various City Council population registries. Families were only included in this study if both parents and twins were willing to cooperate. There were 35 families with MZ boys, 35 with MZ girls, 31 with dizygous (DZ) boys, 30 with DZ girls and 29 with DZ twins of opposite sex.

EDTA blood was obtained between 8.30 and 10.30 a.m. by venepuncture after overnight fasting. Plasma was separated from the cells by centrifugation for 10 min at 3000 rpm. Part of the plasma was kept at 4°C for lipid determinations within the next 5 days. The remainder was stored in 2.5 ml aliquots in tubes with tightly fitting screw-caps at –20°C for later use.

Plasma lipid and (apo)lipoprotein analysis

Cholesterol and triglyceride levels were determined using enzymatic methods (Boehringer, Mannheim, Germany; CHOD-PAP kit no. 236691 and GPO-PAP kit no. 701904). HDL cholesterol was measured following phosphotungstate Mg^{2+} precipitation of VLDL, IDL and LDL according to Lopes-Virella et al. (1977). LDL cholesterol was subsequently calculated using the formula of Friedewald et al. (1972).

Apolipoprotein B was quantified by radial immunodiffusion as described by Havekes et al. (1981). ApoE was quantified by enzyme-linked immunosorbent assays as described by Bury et al. (1986).

Apolipoprotein E phenotyping

The apoE phenotyping was performed using a rapid micromethod based on isoelectric focusing (pH 5–7) of delipidated plasma samples, followed by immunoblotting on nitrocellulose filters using a polyclonal anti-apoE antiserum as previously described (Havekes et al. 1987).

Statistical analysis

The apoE phenotype frequencies and the APOE allele frequencies were determined in the complete set of 160 families with the exception of 2 missing fathers. For the parents and MZ twins, the calculation of the allele frequencies was performed by gene-counting procedures. For the DZ twins, the allele frequencies were estimated according to Martin (1975) using the sib-genotype frequencies of Smith and Penrose (1955).

The MZ intrapair differences were estimated in three different groups: MZ twin pairs with the E3E2 phenotype ($n=12$), MZ pairs with the E3E3 phenotype ($n=39$) and MZ pairs with the E4E3 phenotype ($n=14$). The one MZ pair with the E4E4 phenotype was excluded from these analyses for obvious reasons. We did not observe MZ twin pairs with the E2E2 or the E4E2 phenotypes. The phenotypic difference for each twin pair was calculated as: $d_{f(g)} = y_{gf1} - y_{gf2}$ where the subscript f denotes the twin pair, g denotes genotype, and *1* and *2* denote the first and second twin in the pair with arbitrary labelling. A two-way analysis of variance (ANOVA), with gender and apoE phenotype entered as factors, was then computed for three different estimates of $x_{f(g)}$: (1) calculated according to Magnus et al. (1981) as $x_{f(g)} = |d_{f(g)}|$, (2) calculated according to Elashoff et al. (1991) as $x_{f(g)} = |d_{f(g)} - c_{g\ mean}|$, where c_g denotes the mean genotype-specific difference, and (3) again according to Elashoff et al. (1991), as $x_{f(g)} = |d_{f(g)} - c_{g\ median}|$, where c_g denotes the median genotype-specific difference. In addition, we also followed the approach proposed by Martin et al. (1983) by calculating within-pairs mean squares (WMS) for each trait, followed by a comparison of the WMS for each apoE phenotype by means of Bartlett's test of heterogeneity of variances (Sokal and Rohlf 1981). ANOVA procedures were performed using the programs contained in the statistical package NCSS, version 5.1 (Dr. J. L. Hinze, Kaysville, Utah, USA).

Table 1. Apolipoprotein E phenotype numbers, relative frequencies (in %, in brackets) and apoE allele frequencies in parents and children of 160 Dutch twin families

ApoE phenotype	Parents	Twins	
		MZ[a]	DZ[b]
E2E2	3 (0.9)	0 (0.0)	1 (0.6)
E3E2	48 (15.0)	13 (18.6)	37 (20.6)
E3E3	185 (57.8)	41 (58.6)	88 (48.9)
E4E2	10 (3.1)	0 (0.0)	4 (2.2)
E4E3	67 (20.9)	15 (21.4)	46 (25.6)
E4E4	5 (1.6)	1 (1.4)	4 (2.2)
Total	318	70	180
Alleles:			
E*2	0.101	0.093	0.103
E*3	0.762	0.786	0.726
E*4	0.137	0.121	0.163

[a] Each MZ twin pair is counted as one phenotype
[b] Each DZ twin pair is counted as two phenotypes; for this group, the allele frequencies are calculated from sib-pair phenotype frequencies according to Martin (1975)

Results

Apolipoprotein E phenotype distribution

The apoE phenotype numbers and frequencies for the complete set of parents and twins are presented in Table 1. The observed apoE frequencies in all groups consideres, viz. parents, MZ and DZ twins, were in genetic equilibrium (results not shown). The observed allele frequencies in these three groups do not differ significantly from those found in a larger Dutch population (Smit et al. 1988). There were no significant gender differences with respect to the apoE phenotype distribution in the three groups considered (results not shown).

Influence of apoE polymorphism on intrapair difference in measured parameters in MZ twins

The influence of the apoE polymorphism on the plasma lipid levels was estimated by comparing the intrapair differences in the levels of the quantitative traits in the MZ twins, initially as outlined by Magnus et al. (1981). As explained above, we only considered the MZ twin-pairs with the E3E2, E3E3 or E4E3 phenotypes. As shown in Table 2, the mean apoE phenotype-specific intrapair differences for each of the traits, estimated by $x_{f(g)} = |d_{f(g)}|$ (see statistical analysis for further details), did not differ significantly. However, since it was reported that this approach could result in Type I or Type II errors (Browne and Forsythe 1974), we repeated our calculations, following the suggestions of Elashoff et al. (1991), using a Levene test. This involves the subtraction of a measure of central tendency from the phenotypic difference for each twin pair (see statistical analysis for further details). We considered two different estimates of central tendency c_g, with c_g representing either the mean genotype-specific difference or the median genotype-specific difference. However, as can

Table 2. ApoE genotype-specific mean within-pair differences ($x_{f(g)}$) ± SD estimated by three different methods for MZ

	ApoE phenotype			P^a
	E3E2 (n = 12) $x_{f(g)}$ ± SD	E3E3 (n = 39) $x_{f(g)}$ ± SD	E4E3 (n = 14) $x_{f(g)}$ ± SD	
$x_{f(g)} = \lvert d_{f(g)} \rvert$				
Cholesterol[b]	0.45 ± 0.31	0.37 ± 0.31	0.26 ± 0.30	NS
Triglycerides	0.25 ± 0.14	0.12 ± 0.19	0.18 ± 0.15	NS
LDL-cholesterol	0.38 ± 0.28	0.31 ± 0.25	0.26 ± 0.26	NS
ApoB	10.2 ± 7.6	6.6 ± 7.5	5.9 ± 7.5	NS
ApoE	1.1 ± 1.0	1.0 ± 0.6	0.8 ± 0.8	NS
$x_{f(g)} = \lvert d_{f(g)} - mean\ d_{f(g)} \rvert$				
Cholesterol[b]	0.38 ± 0.28	0.36 ± 0.25	0.25 ± 0.26	NS
Triglycerides	0.23 ± 0.14	0.11 ± 0.12	0.17 ± 0.15	NS
LDL-cholesterol	0.36 ± 0.24	0.31 ± 0.25	0.24 ± 0.30	NS
ApoB	9.7 ± 6.9	6.6 ± 6.9	6.3 ± 7.1	NS
ApoE	1.1 ± 0.7	1.0 ± 0.6	0.8 ± 0.7	NS
$x_{f(g)} = \lvert d_{f(g)} - median\ d_{f(g)} \rvert$				
Cholesterol[b]	0.38 ± 0.28	0.36 ± 0.31	0.25 ± 0.30	NS
Triglycerides	0.23 ± 0.14	0.11 ± 0.12	0.17 ± 0.15	NS
LDL-cholesterol	0.35 ± 0.24	0.31 ± 0.25	0.24 ± 0.26	NS
ApoB	9.5 ± 7.3	6.6 ± 7.5	5.8 ± 7.5	NS
ApoE	1.1 ± 1.0	1.0 ± 0.6	0.8 ± 0.7	NS

NS, not significant ($P > 0.05$)

[a] P value indicating the difference between the apoE phenotpye groups calculated by means of two-way ANOVA with apoE phenotye and gender entered as factors

[b] Levels are expressed in mmol/l except for the apoB and apoE levels, which are expressed in mg/100 ml

Table 3. Within-pairs mean squares (WMS) by apoE phenotype for levels of plasma lipids and (apo)lipoproteins in MZ twins

	E3E2 (n = 12)	E3E3 (n = 39)	E4E3 (n = 14)	χ^{2a}
Cholesterol	0.33	0.23	0.10	4.01
Triglycerides	0.09	0.04	0.06	4.77
LDL cholesterol	0.26	0.16	0.10	2.73
ApoB	189.30	76.10	115.20	4.06
ApoE	2.09	1.87	0.84	2.92

[a] χ^2 and its significance were calculated by means of Bartlett's test of heterogeneity of variances. The level of χ^2 is significant larger than 5.99 (with $df = 2$)

be inferred from Table 2, this did not result in a drastic change in the phenotype-specific mean intrapair differences. For each of the traits, these models did not reveal any significant difference between the three apoE phenotype groups, as evaluated by two-way ANOVA. In addition, there was (1) no significant influence of gender on the within-pair differences, and (2) no significant interaction between apoE phenotype and gender (results not shown). We also followed a different approach, suggested by Martin et al. (1983), and used the WMS as an estimate for the intrapair difference. However, this approach also did not result in any significant apoE phenotype-specific mean intrapair difference (Table 3). Altogether, our results indicate that, in the MZ twins that we studied, no significant interaction between environmental variability and apoE phenotype for any of the lipid traits considered could be detected.

Discussion

In the present study, we describe the apoE phenotype distribution and APOE allele frequencies for the parents, and MZ and DZ twins from 160 Dutch twin families. The apoE phenotype distribution and APOE allele frequencies in each of the three groups were comparable with the values reported previously for a large Dutch population (Smit et al. 1988).

In various studies, the apoE polymorphism has been reported to influence plasma lipid and (apo)lipoprotein levels, although to a variable extent (Davignon et al. 1988). The APOE*4 allele was found to be associated with increased levels of total plasma cholesterol, LDL cholesterol, apoB and recently also with increased levels of Lp(a) (de Knijff et al. 1991), whereas the opposite holds true for the APOE*2 allele. Conversely, in APOE*4 allele carriers, plasma apoE levels were decreased, whereas the apoE levels were increased in APOE*2 allele carriers. The mechanism behind the effect of the apoE polymorphism on plasma (apo)lipoprotein levels is commonly assumed to be the result of the influence of the apoE polymorphism on the efficiency of the catabolism of chylomicron and VLDL remnants (Utermann 1985; Weintraub et al. 1987; Demant et al. 1991). These influences were also found by us for this group of twin families (Kempen et al. 1991).

Several studies have provided evidence for a significant interaction between environmental (e.g. dietary) variability and apoE polymorphism (Miettinen et al. 1988, 1992; Tikkanen et al. 1990a; Manttari et al. 1991). These studies demonstrate that individuals carrying an APOE*4 allele show a greater sensitivity for dietary interventions when compared with individuals without this allele. This interaction could, at least partly, be explained by differences in intestinal cholesterol absorption and cholesterol synthesis (Miettinen 1991; Miettinen et al. 1992). However, similarly convincing studies have failed to detect any interaction between the apoE polymorphism and dietary variability (Fisher et al. 1983; Savolainen et al. 1991; Glatz et al. 1991). Furthermore, Hallman et al. (1991) showed that the influence of the apoE polymorphism on plasma cholesterol levels in nine different populations was remarkable consistent, despite marked differences in apoE phenotype frequencies and dietary habits between the various populations. This suggests that the APOE gene influences the plasma cholesterol levels independent of environmental factors.

We speculate that these conflicting results can be, at least in part, explained by the presence of genetic differences between the various population. This could give rise to gene-gene and gene-environment interactions to a variable extent in these populations of unrelated individuals. In two studies, Pedersen and Berg (1989, 1990) reported an interaction between the *Pvu*II restriction fragment

length polymorphism (RFLP) at the LDL receptor locus and apoE in two Norwegian populations. They found that the increased plasma cholesterol and LDL cholesterol levels associated with apoE4 are reduced, by an unknown mechanism, in those individuals with the *Pvu*II-A1 allele. Conversely, in a Finish population, the raised LDL cholesterol levels in individuals with apoE4 are further increased by the X*2 allele of the *Xba*1 RFLP in the APOB gene (Aalto-Setälä et al. 1988; Miettinen 1991). In addition, there are studies suggesting that genetic variability in the APOB gene interacts with dietary variability (Tikkanen et al. 1990b; Abbey et al. 1991).

Instead of studying associations in randomly selected populations of unrelated individuals, Magnus et al. (1981) were the first to suggest the use of mean genotypic intrapair differences, estimated in MZ twin pairs, as a means of examining gene-environment interactions without the disturbing effects of gene-gene interactions (MZ twins share by definition the same nuclear genes). Using this approach, Berg (1987) showed, for example, a significant interaction between environmental variability and the APOB gene on the plasma levels of apoB. The results of the present study indicate that the intrapair differences in the group of MZ twins were not significantly different between the three apoE phenotype groups (Table 2). However, since absolute differences can have highly skewed distributions and this, in its turn, can affect both type I and Type II errors in subsequently performed tests (Browne and Forsythe 1974), we calculated our results as proposed by Martin et al. (1983) and Elashoff et al. (1991). This resulted in similar results, sustaining our initial observations, and indicated that the influence of the APOE gene on plasma lipids is not affected by environmental variability. This identifies the APOE gene as a "level" gene according to the definitions proposed by Magnus et al. (1981) and Berg (1984, 1987, 1990).

The twins that we studied were young and still living with their parents. As was pointed out by Magnus et al. (1981), intrapair difference may be more pronounced in older twins or in twins not living together. Likewise, higher intrapair differences can be expected between twins living separately. Some evidence for this was published by Koskenvuo et al. (1989). They showed that apoE phenotype-specific mean intrapair differences were approximately twice as high in MZ twins reared apart when compared with the differences in MZ twins living together. Therefore, we have recently started a second study among older twins not living together, allowing for maximum environmental variability.

In summary, our results indicate that, in our sample of adolescent MZ twins, the APOE gene acts as a "level" gene, i.e. a gene with an direct influence on plasma lipid and (apo)lipoprotein levels. This supports the hypothesis that the apoE polymorphism plays an important and independent role in determining plasma lipid levels.

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